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Pattern of reserve storage of the two mussel species *Perna* perna and Mytilus galloprovincialis living on Moroccan coasts: annual variation and effect of pollution

Patrones de almacenamiento de reservas en dos especies de mejillón *Perna perna* y *Mytilus galloprovincialis* de las costas de Marruecos: variación anual y efecto de la contaminación

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Recibido el 23-XI-2005. Aceptado el 30-X-2007

ABSTRACT

The pattern of reserve storage and its importance in the reproductive cycle of the two mussel species living in Moroccan coasts, Perna perna and Mytilus galloprovincialis, were studied comparatively, during two annual cycles. Study was conducted at a polluted and an unpolluted site in Agadir bay. Stereological analysis shows that P. perna presents only one type of storage cells (vesicular cells) storing glycogen. In M. galloprovincialis, two types of storage cells are present: vesicular cells and adipogranular cells (containing glycogen, lipids and proteins). In both species, seasonal variations of reserve tissue volume are conversely proportional to those of the germinal tissues. Reserve tissue appeared in spring (May) and increased in summer. It decreased quickly from August onwards, and disappeared between January and April. In M. galloprovincialis, adipogranular cells disappeared before vesicular cells and reappeared first. In the polluted site, seasonal variation of reserve and germinal tissues is comparable to that in unpolluted site but the maximum value of reserve tissue percentage is less important. Moreover, during the year, this tissue did not disappear. Biochemical analysis shows that mean values of glycogen and proteins quantities are significantly higher in P. perna than in M. galloprovincialis. However, total lipids quantity mean is higher in M. galloprovincialis. Seasonal variations of the three biochemical parameters present a similar profile in the two species. Compared to unpolluted site animals, in polluted one, molluscs possess low glycogen and high lipids levels. Seasonal variations of these parameters show a perturbed profile.

RESUMEN

Se ha estudiado y comparado el patrón de almacenamiento de reservas y su importancia en el ciclo reproductor en dos especies de mejillón de las costas de Marruecos, *Perna perna* y *Mytilus galloprovincialis*, durante dos ciclos anuales. Este estudio se llevó a cabo en un sitio contaminado y otro no contaminado de la bahía de Agadir. El análisis estereológico demostró que *P. perna* presenta un solo tipo de células de reserva (células vesiculares) almacenando glucógeno. En *M. galloprovincialis*, hay dos tipos de células de

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reserva: células vesiculares y células adipogranulares (conteniendo glucógeno, lípidos y proteínas). En ambas especies, las variaciones estacionales del volumen de tejido de reserva son inversamente proporcionales a las del tejido germinal. El tejido de reservas apareció en la primavera (mayo) y se incrementó en verano. A partir de agosto, disminuyó rápidamente y desapareció entre enero y abril. En M. galloprovincialis, las células adipogranulares desaparecieron antes que las células vesiculares y son las primeras en aparecer en el siguiente ciclo. En el sitio contaminado, la variación estacional de tejidos de reserva y germinales es similar a la del sitio no contaminado, salvo que el porcentaje máximo de tejido de reserva es menor. Además, este tejido no desapareció a lo largo del año. Los análisis bioquímicos muestran que las cantidades promedias de glucógeno y de proteínas son significativamente más altas en P. perna que en M. galloprovincialis. Sin embargo, el promedio de lipidos totales fue mayor en M. galloprovincialis. Las variaciones estacionales de los tres parámetros presentan un perfil similar en las dos especies. Comparados con los del sitio no contaminado, los animales del sitio contaminado poseen niveles bajos en glucógeno y altos en lípidos. Las variaciones estacionales de estos parámetros muestran un perfil perturbado.

KEY WORDS: Agadir, Morocco, mussels, *Mytilus galloprovincialis, Perna perna*, pollution effects, reproductive cycle, reserves strategy.

PALABRAS CLAVE: Agadir, Marruecos, mejillones, *Mytilus galloprovincialis, Perna perna*, efectos de la contaminación, ciclo reproductor, estrategias de reserva.

INTRODUCTION

Two sympatric mussel species are found along the Moroccan coast: the African mussel Perna perna and the Mediterranean mussel Mytilus galloprovincialis. The first species is located in the north of its geographical distribution; the second one in its southern limit. In Agadir bay, these local populations of mussels have important commercial values (ID HALLA, 1997) and environmental interest (NAJIMI, BOUHAIMI, DAUBÈZE, ZEKHNINI, PEL-LERIN, NARBONNE, AND MOUKRIM, 1997; KAAYA, NAJIMI, RIBERA, NARBONNE, AND Moukrim, 1999; Moukrim, Kaaya, NAJIMI, ROMÉO, GNASSIA-BARELLI, AND NARBONNE, 2000) as sentinel species.

The few studies carried out on mussel biology in this area, concern only some aspects of the reproduction cycle (SHAFEE, 1989; ID HALLA, BOUHAIMI, ZEKHNINI, NARBONNE, MATHIEU, AND MOUKRIM, 1997). They showed that *P. perna* and *M. galloprovincialis* present synchronous reproductive cycles, and breed throughout the year with a principal spawning period in spring. The only differences observed by ID HALLA *ET AL*. (1997), regard the duration of the principal spawning period (more important for *P. perna*) and the genital activity rhythm in summer (reduced in this species).

In view of the importance of reserves in the reproductive cycle (BAYNE, BUBEL, GABBOTT, LIVINGSTONE, LOWE AND MOORE, 1982; LOWE, MOORE AND BAYNE, 1982; PIPE, 1987) and of the particularities of reserve strategy in Mytilidae (strategy essentially based on the glycogen, presence of specific storage cells and existence of a direct relationship between reserve metabolism and reproductive cycle), it was interesting to conduct a comparative study of *P. perna* and *M. galloprovincialis* in order to describe the reserves strategy for these two species living in the same environmental conditions. The two molluscs present an important difference regarding the cells involved in the reserve storage. As indicated by Lunetta (1969) P. perna presents only one type of storage cells (vesicular cells or glycogen cells), storing especially the glycogen ; however, in M. galloprovincialis (HERLIN-HOUTTEVILLE, 1974; DAN-TON, KIYMOTO, KOMARU, WADA, AWAJI AND MATHIEU, 1996), two types of storage cells are present, vesicular cells (storing glycogen) and adipogranular cells (containing glycogen, lipids and proteins).

Furthermore, beyond the determination of pattern of reserve storage and its importance in the reproductive cycle of the two mussels living in Moroccan coasts, this work studies the impact of pollution on this pattern. Therefore, a comparative study of two sites (unpolluted and polluted) was conducted.

MATERIAL AND METHODS

Sampling

This study was conducted during annual reproductive cvcles two (October 1994 to August 1996). Two types of sites, representative of the Agadir marine bay were considered: i) a reference site (unpolluted), Cap Ghir, located 50 km north of Agadir City and far from any human activity, and ii) a polluted site (Anza) located 5 km north of Agadir and receiving the industrial and domestic untreated waste waters of Anza zone. Many studies, conducted during the same period of the present study, showed that this site is contaminated by heavy metals and PAHs either accumulated by mussels or in sediment Halla, 1997; Najimi, 1997; (ID MOUKRIM, KAAYA, NAJIMI, ROMÉO, **GNASSIA-BARELLI AND NARBONNE 2000;** KAAYA, 2002), with some perturbations in the physical and chemical parameters of seawater (ID HALLA, 1997).

During this study, for each month, ninety individuals of 35 mm for each species are collected at random in each of the two sites. 30 individuals/ species/sites were subject to stereological analysis and 60 individuals/ species/sites were dedicated to the biochemical analysis.

Stereological analysis

In order to follow the seasonal variation of the glycogen according the reproduction cycle in Mytilus galloprovincialis and Perna perna, an histochemical study (stereological analysis) was conducted in the mantle. Thirty adult mussels were collected and directly fixed in Gendre liquid for 3 days. Animals were then removed from shells and a central portion of mantle tissue was correctly excised and embedded in paraffin. Sections of 6 µm were cut and stained by the periodic acid of Schiff (PAS). Stereological analysis was applied according the method of WEIBEL, KISTLER, AND SCHERLE (1966) which quantified the volume occupied by the reserve and germinal tissues. The cell types were determined according the definition of LUBET (1959): i) adipogranular cells (20-25 µm for length, 4.5-5 µm for length of nucleus, green coloration, contain lipids, proteins and glycogen), and *ii*) vesicular cells (50-80 µm for length, 3-5 µm for length of nucleus, pink coloration, contain only glycogen)

Biochemical analysis

Biochemical analysis (glycogen, lipids and proteins) was carried out on mussel mantle which in Mytilidae, constitutes an important organ in storage of reserves. Monthly, sixty adults were randomly sampled, quickly transferred to the laboratory in isotherm conditions and frozen at -30°C. Glycogen, total lipids and proteins were respectively measured according the methods of DUCHATEAU AND FLORKIN (1959), FOLCH, LEES AND SLOANE-STANLEY (1957) and LOWRY (1951). Rates of these compounds were expressed as mg/g fresh weight (mg/g FW).

Statistical analysis

Stereological analysis was expressed as a percentage of the total volume of mantle tissue ; biochemical results as means ± standard deviations. The statistical significance of difference between samples was evaluated by the "t" test using the Statistica software (Release 4.5A Statsoft Inc. Ed. 1993). A "P" value of less than 0.05 was considered as statistically significant.

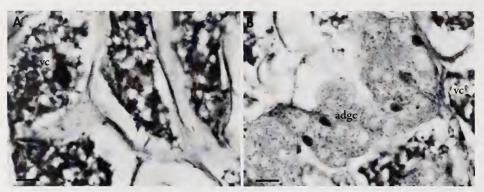


Figure 1. Cells implicated in the reserve storage in *Perna perna* (A) (only one type of cells: vc: vesicular cells) and *Mytilus galloprovincialis* (B) (two types of cells, vc: vesicular cells; adgc: adipogranular cells). Scale bars 10 µm.

Figura 1. Células implicadas en la acumulación de reservas en Perna perna (A) (solo un tipo de células: vc: células vesiculares) y Mytilus galloprovincialis (B) (dos tipos de células, vc: células vesiculares; adgc: células adipogranulares). Escalas 10 µm.

RESULTS

Stereological analysis

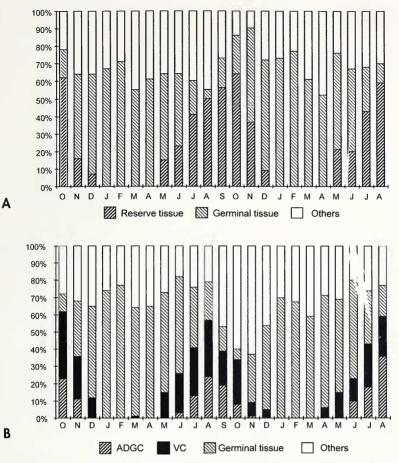
Figure 1A shows that the mantle of *Perna perna* presents only one type of storage cells: vesicular cells (VC). In *Mytilus galloprovincialis* (Fig. 1B), two types of storage cells are present: adipogranular cells (ADGC) and vesicular cells (VC).

For *P. perna*, the respective volumes of germinal and reserve tissues are conversely proportional (Fig. 2A). The seasonal profile is similar during the two studied annual cycles. The volume of germinal tissue increases from October to February, when the maximum is reached (respectively 72% and 77% in the first and second year). From March onwards, the germinal tissue volume decreases to reach the minimum in August (respectively 8% and 6% in the first and second year). The reserve tissues appeared in spring (May) and increased until reaching maximal values in summer (August). They decreased quickly and disappeared between January and April. In the polluted site (Fig. 3A), the seasonal variation of reserve and germinal tissues is similar to that in the reference site but the maximum value of reserve tissues percentage is lower (57% and 62% respectively in the first and second year). Moreover, during the year, this tissue did not disappear. The minimum value recorded was 7-10% in February.

For Mytilus galloprovincialis (Fig. 2B), the volume of germinal tissue increases in autumn and in the beginning of winter to reach a maximum in February (75%). Thereafter, we observe a reduction of the surface occupied by this tissue until October where the minimum (12%) is reached. During the second cycle a similar evolution is recorded. Otherwise, as with P. perna, the volume of germinal tissue is inversely proportional to reserve tissue. Nevertheless, in this case, the maximum is reached in October (64%), whereas the disappearance takes place from January to March for the two studied yearly cycles. In the polluted site (Fig. 3B), the reserve tissue presents a seasonal evolution similar to the reference site. Besides, this tissue never disappears as in P. perna. The occupied minimal volume is 5% reached in February.

Moreover, for *M. galloprovincialis*, in the reference site, the adipogranular cells disappeared before the vesicular cells and then reappeared first. In the polluted site the two cellular categories persist

MOUKRIM ET AL.: Reserves strategy of mussels in Moroccan coasts



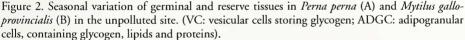


Figura 2. Variación estacional de los tejidos germinal y de reservas en Perna perna (A) y Mytilus galloprovincialis (B) en el sitio no contaminado. (VC: células vesiculares almacenando glucógeno; ADGC: células adipogranulares, conteniendo glucógeno, lípidos y proteínas).

during all the year. However, an oscillation can be observed in the volumes occupied by the two categories of cells.

Biochemical analysis

Glycogen: The mean amount of glycogen is significantly (F= 11.19 and P= 0.027) higher in *Perna perna* than in *Mytilus galloprovincialis* (respectively 59.6 \pm 7.36 and 48.7 \pm 4.78 mg/g FW). For the two species, the seasonal variations are significant (F= 4.95; P= 10⁻⁶ for

Perna perna and F= 2.65; P= 4.7x10⁻⁶ for *Mytilus galloprovincialis*) and exhibit a similar profile (Fig. 4A). The glycogen increases in spring (from April), reaches a maximal value in August (219.4 and 191.9 mg/g FW in *Perna perna* and *Mytilus galloprovincialis* respectively), then decreases during autumn and winter. The minimal values are noted in February (0.39 and 0.34 mg/g FW in *Perna perna* and *Mytilus galloprovincialis* respectively).

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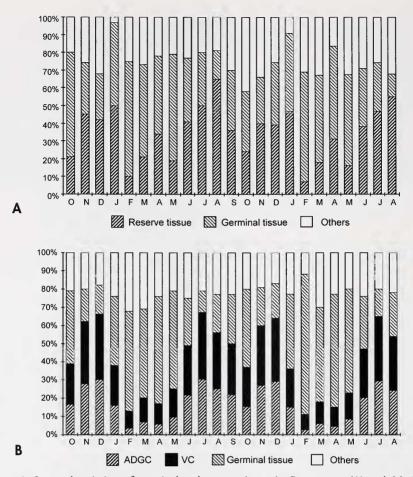


Figure 3. Seasonal variation of germinal and reserve tissues in *Perna perna* (A) and *Mytilus gallo-provincialis* (B) in the polluted site. (VC: vesicular cells storing glycogen; ADGC: adipogranular cells, containing glycogen, lipids and proteins).

Figura 3. Variación estacional de los tejidos germinal y de reservas en Perna perna (A) y Mytilus galloprovincialis (B) en el sitio contaminado. (VC: células vesiculares almacenando glucógeno; ADG: células adipogranulares, conteniendo glucógeno, lípidos y proteínas).

In the polluted site (Fig. 4B), the difference between the mean amount of glycogen in the two species (39.75 and 23.44 mg/g FW in *P. perna* and *Mytilus galloprovincialis* respectively) is not significant (F= 6.257 and P= 0.130). Otherwise, their seasonal variation are significantly different (F= 4.03 and P= 10^{-6} in *P. perna* and F= 9.78 and P= 10^{-7} in *M. galloprovincialis*). The seasonal profile is completely different for the two molluscs: For *P. perna*, it presents three peaks, the first in June (96.05 mg/g FW), the second in November (118.87 mg/g FW) and the third in May of second year (159.02 mg/g FW). For *M. galloprovincialis*, only two peaks are recovered 65.03 and 153.64 mg/g FW respectively in May and November of the first year.

The comparison of values for reference and polluted site animals shows a significant difference. The values in the polluted site are less important (39.8 \pm 13.36 instead of 59.6 \pm 7.36 mg/g FW in

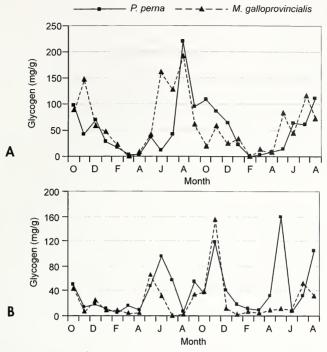


Figure 4. Seasonal variation of glycogen in the mantle of *Perna perna* and *Mytilus galloprovincialis* in the reference site (A) and the polluted site (B)

Figura 4. Variación estacional del glucógeno el el manto de Perna perna y Mytilus galloprovincialis *en el sitio de referencia (A) y en el sitio contaminado (B)*

the reference site, for *Perna perna*). Moreover, the seasonal profile of glycogen content (Fig. 4B) presents some perturbations compared to the reference site.

Lipids: A significant difference was noted between the mean amounts of total lipids in the two mussels species (92.86 and 125.04 mg/g FW in P. perna and Mytilus galloprovincialis respectively). However, the difference of their seasonal variation are significant with F= 4.42 and $P = 10^{-6}$ in *P. perna* and F = 3.6 and P =4.5x10⁻⁵ in *M. galloprovincialis*. In the first year, the seasonal profile (Fig. 5A) is similar for the two molluscs. The lipid content increased in autumn, with a maximal value in December (respectively 354.3 and 316.9 mg/g FW in Perna perna and Mytilus gal*loprovincialis*), and decreased in winter and spring. However, in the second year, a slight increase was noted in summer (June - July) only in P. perna.

In the polluted site, the two species present a significant difference between total lipids mean values (respectively 231.4 \pm 21.47 and 135.5 \pm 16.93 mg/g FW in *Perna perna* and *Mytilus galloprovincialis*). Besides, the difference in their seasonal variation (Fig. 5B) is significant with F= 2.59 and P= 0.002 in *P. perna* and F= 3.60 and P= 4.5x10⁻⁴ in *M. galloprovincialis*.

Otherwise, the values are generally higher for the polluted site than the reference site for the two species. Moreover, compared to the unpolluted site, the seasonal variation (Fig. 5B) shows a perturbed profile with several peaks during the annual cycle and a maximum values is reached rather in August (777.03 mg/g FW in *P. perna* and 659.95 mg/g FW in *M. galloprovincialis*).

Proteins: The mean content of protein is significantly higher in *Perna perna* (28.9

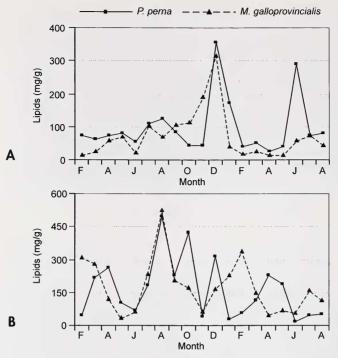


Figure 5. Seasonal variation of lipids in the mantle of *Perna perna* and *Mytilus galloprovincialis* in the reference site (A) and the polluted site (B).

Figura 5. Variación estacional de los lípidos en el manto de Perna perna y Mytilus galloprovincialis en el sitio de referencia (A) y en el sitio contaminado (B).

 \pm 3.51 mg/g FW) compared to *Mytilus* galloprovincialis (20.2 \pm 3.17 mg/g FW). However, the seasonal profile (Fig. 6A) is similar for the two species. The maximal values were generally recorded in summer and autumn in the first year and in winter and beginning spring in the second year, whereas the minimal values were reached at the end of autumn and the beginning of winter.

In the polluted site (Fig. 6B), the mean amount of protein is higher in *P. perna* ($30.11 \pm 7.742 \text{ mg/g FW}$) than in *M. galloprovincialis* (19.74 mg/g FW). However, the mean values and the seasonal profile do not show any significant difference compared with those observed from the reference site (F= 1.08 and P= 0.30 for *Perna perna*; F= 0.31 and P= 0.57 for *Mytilus galloprovincialis*). For the two species, the maximum values were recorded in summer and autumn.

DISCUSSION

The comparative study of the reserve pattern of the two mussels species living in the Moroccan coasts, *Perna perna* and *Mytilus galloprovincialis*, sampled in the same site (Cap Ghir, Agadir bay), shows that, in spite of the difference in their reserve tissues (only one cell type, vesicular cells, in *Perna perna*; two cell types, adipogranular cells and vesicular cells, in *Mytilus galloprovincialis*), the two molluscs have a similar fluctuation.

This strategy presents cycles (of reserve compounds and cells involved in the storage) which, compared to the cycle of reproduction (determined in these same animals by ID HALLA *ET AL.*, 1997) are inversely proportional to it. The accumulation of reserves in these cells is related to the period of reduced sexual

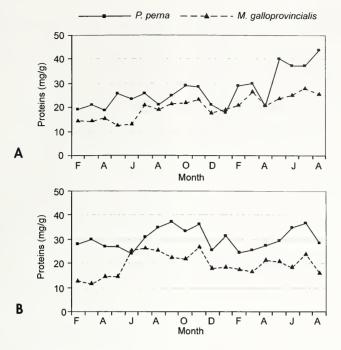


Figure 6. Seasonal variation of proteins in the mantle of *Perna perna* and *Mytilus galloprovincialis* in the reference site (A) and the polluted site (B).

Figura 6. Variación estacional de las proteínas el manto de Perna perna y Mytilus galloprovincialis en el sitio de referencia (A) y en el sitio contaminado (B).

activity (in summer). The low levels of reserve are obtained in autumn and particularly in winter (gametogenesis and spawning periods). Similar results have been reported in Perna perna from Brazilian coasts (LUNETTA, 1969) and in Mytilus galloprovincialis from the French coasts (HERLIN-HOUTTEVILLE, 1974: DANTON, KIYMOTO, KOMARU, WADA, AWAJI AND MATHIEU, 1996). Also, in the Ria de Vigo (Spain), the profile of the variations of the gonadic index and somatic index in cultured mussels show clearly their inverse and gradual fluctu-(CÁCERES-MARTÍNEZ ations AND FIGUERAS, 1998). This association between gonad and storage tissue cycles in mussels is well known. Decline of the ADG cells occurs during gametogenesis by a lysosomal autophagic mechanism (BAYNE ET AL., 1982; LOWE, MOORE AND BAYNE; 1982; PIPE, 1987). The energy

used in gonad restoration following spawning during spring and summer probably derived directly from feeding since ADG cells disappeared from the mantle in early spring (VILLALBA, 1995).

Furthermore, the reserve accumulation in the two mollusc species is related to the proliferation of the phytoplanctonic biomass linked to upwelling currents which take place, between February and August, in the Agadir bay and more precisely in Cap Ghir (BELVEZE, 1984 ; Agoumi and Orbi, 1992). Cáceres-Martínez AND FIGUERAS (1998) reported that this increase in food availability for mussels in the area favours the accumulation of reserves during this period. These results also confirm the close dependence, described by MATHIEU (1987), between the reserve storage and environmental conditions in marine ecosystems. As suggested by

this author, the disappearance of reserve tissue in mussels seems to be under endocrine control via cerebroid ganglia which provoke disappearance of adipogranular cells and vesicular cells in the mantle after liberation of their reserves which are indispensable for gametogenesis and spawning process. The synchronic character of this disappearance, in *Perna perna* and *Mytilus galloprovincialis,* could be explained by the existence of the same mechanism for the control of reserves in both species.

The comparison of the reserve compounds in the two molluscs shows that the glycogen, total lipids and proteins contents are higher in Perna perna (in spite of the presence of only one type of reserve storage cells, vesicular cells) compared to Mytilus galloprovincialis. These results could explain the differences observed by ID HALLA ET AL. (1997) between the reproductive cycles of these bivalves. According to these authors, in Perna perna, compared to Mytilus galloprovincialis, the mainspawning period in spring is longer and the sexual activity in summer is reduced. Otherwise, the important reserve levels in this species could be attributed to the spatial distribution of each species: Perna perna lives essentially at the infra-littoral level and is more immersed than Mytilus galloprovincialis (ID HALLA ET AL., 1997) and, consequently, has access to more nutrients, thus allowing the synthesis and storage of more reserves. SEED (1976) showed that the gonadal development was faster in mussels from the low intertidal zone, than in those from the upper zone and related these results to food availability. Other studies associated local variations in gonadal cycle with environmental conditions (FERRÁN, 1991; VILLALBA, 1995). According to CÁCERES-MARTÍNEZ AND FIGUERAS (1998), there is no influence of locality and depth in the gonadal development of cultured mussels.

The seasonal profile of the biochemical reserves studied shows that the first peak of glycogen and lipids, recorded in summer, is related to the occurrence of upwelling currents which provide food availability, ensuring an abundant planktonic food supply for mussels. According to CACERES-MARTÍNEZ AND FIGUERAS (1998), massive spawns occur in spring coinciding with an increase in temperature and chlorophyll-a concentration in the area providing favorable conditions for larval growth. In winter, the second peak of lipids coincides with the maturity period of gametes. This peak takes place just after the disappearance of the glycogen peak. It is probably a result of the glycogen transformation. The metabolic conversion of glycogen to lipids has been reported by Zaba and DAVIES (1980), using ¹⁴Cglucose. According to GOSLING (1992), the mantle is considered as the organ of many and extensive metabolic transformations during the sexual cycle. The reserves, particularly the glycogen, accumulated during summer, are used in autumn and winter for the gametogenesis. Similar results were reported by SHAFEE (1989) in Perna picta of Temara (North of Morocco).

In the polluted site (Anza), many perturbations in the metabolism of reserves were noted comparatively to the reference site (Cap Ghir). The reserve tissues, which disappeared in winter and at the beginning of spring, in mussels of Cap Ghir, persist throughout the sexual cycle in Anza mussels. This could probably be a result of the pollution effect on the cerebroid ganglions neurosecretions which are, according to LUBET, HERLIN, MATHIEU, AND COLLIN (1976) and MATHIEU (1987) involved in the control of reserve cells.

The analysis of the seasonal profile of the reserve levels in mussels sampled in the polluted site shows some perturbations as compared to the reference site. For example, the glycogen content is low in summer (August), in spite of the availability of phytoplanctonic biomass in this period. This is probably linked to the stress caused by pollution of industrial and domestic waste waters discharged directly in this site, without any treatment. This fact was indicated by DESLOUS-PAOLI, WOLOWICZ, AND BOROMTHANARAT (1991) who reported that, in *Mytilus edulis*, reserves could be used both in reproductive process and to overcome the hard environmental conditions. According to THOMPSON (1972) the reserves are used in order to reach the basal level of energy necessary for stressed animals.

Contrary to the glycogen, the total lipids are more important in *Perna perna* and *Mytilus galloprovincialis* living in Anza (polluted site). This could be explained by an eventual direct assimilation of lipids from the organic matter of waste waters and/or a change of the reserve storage process. According to GOSLING (1992) the lipid storage process in molluscs is considerably linked to the environmental conditions, particularly to the presence of pollutants.

As a general conclusion, in the two species of mussel *P. perna* (with only vesicular cells) and *M. galloprovincialis* (with adipogranular cells and vesicular cells), the respective germinal and reserve tissues clearly show their inverse and gradual profile. Their seasonal fluctuations are similar in the two molluscs. In the polluted site, many perturbations of the reserve metabolism were noted comparatively to the reference site. Then, contrary to animals of this latter site, which presented a glyco-

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genic strategy, a lipidic strategy takes place in molluscs of the polluted site.

The results obtained in this work constitute a contribution to the knowledge of the reserve strategy in the two species living along Moroccan coasts, the African mussel Perna perna and the Mediterranean mussel Mytilus galloprovincialis. So, several facts are reported: i) the type, the seasonal variation and the cycle of the reserve strategy in the two molluscs, ii) cells involved in the storage, iii) the relationship between the reserve strategy and the reproduction cycle, and iv) the response of this strategy to the environmental conditions. Nevertheless, it would be interesting to identify the mechanism and the control process of the reserve strategy in these molluscs.

ACKNOWLEDGMENTS

We are grateful to Miss Joanne Preston (Southampton University, UK) and Mrs Barbara Picot (Angers University, France) for their Language corrections. We thank the IFS (Sweden), AUPELF (Canada), Ministère des Affaires Etrangères (France) and Ministère de l'Enseignement Supérieur et de la Recherche Scientifique (Morocco), for their financial assistance.

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